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Stephen M. Griffith  
*Utah State University*

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THE EFFECTS OF  $\text{SO}_2$  ON  $\text{N}_2$ -FIXATION, CARBON PARTITIONING,  
AND YIELD COMPONENTS IN SNAPBEAN, PHASEOLUS VULGARIS L.

by

Stephen M. Griffith

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

UTAH STATE UNIVERSITY  
Logan, Utah

1983

DEDICATION

To my Carol Ann

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. William F. Campbell for the use of his facilities and for his encouragement and advice throughout this project.

Special appreciation is also extended to Dr. Roger E. Wyse for his valuable advice, interest, and confidence in my abilities.

I would also like to thank Dr. Jaleh Daie for her support and encouragement but most of all for the additional assistance preparing the manuscript for this thesis.

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My deepest appreciation goes to my mother, grandmother, and sisters for their continued love and support.

Above all I wish to express my heartfelt thanks to my lovely wife, Carol, for her love, faith, and special friendship she unselfishly extends. To my sons, Stephen and David, I express my love and thanks for their understanding when this project demanded that I spend additional time away from home.

*Stephen M. Biffert*



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## ABSTRACT

The Effects of  $\text{SO}_2$  on  $\text{N}_2$ -fixation, Carbon Partitioning and  
Yield Components in Snapbean, Phaseolus vulgaris L.

by

Stephen M. Griffith, Master of Science

Utah State University, 1983

Major Professor: Dr. William F. Campbell  
Department: Plant Science

The primary air pollutant sulfur dioxide has been shown to affect plant biochemistry and physiology, although very little is known about its effects on  $\text{N}_2$ -fixation in legumes.

This study was designed to determine if  $\text{N}_2$ -fixation, carbon partitioning, and productivity are affected under short term low level,  $\text{SO}_2$  exposures.

Greenhouse grown snapbeans (Phaseolus vulgaris L. cv. Earliwax), 29 days from planting, were exposed to 0.0, 0.4, and 0.8 parts per million sulfur dioxide for 4 hours  $\text{day}^{-1}$  for 5 days in a fumigation chamber. At these concentrations there was no visible damage of the plant tissue and no significant changes in dry weight or yield components. Only the 0.8 parts per million sulfur dioxide treatment reduced acetylene reduction rates but rates returned to control levels within 2 days after the removal of the stress. Sulfur dioxide treatment increased the total carbon-14 exported from the leaves of 0.4 parts per million sulfur dioxide treated plants while the 0.8 parts per million sulfur dioxide treated plants were found to retain more of their total carbon-14. This retention of carbon-14 at the 0.8

parts per million level may account for the inhibition of acetylene reduction due to lower photosynthate supplies arriving at the root-nodules.

These data suggest that low sulfur dioxide levels that would not cause any visible injury, may be interacting with carbohydrate assimilation and/or transport in P. vulgaris.

(57 pages)

## INTRODUCTION

### Sulfur Dioxide Pollution

Sulfur dioxide ( $\text{SO}_2$ ), a colorless, pungent gas, is one of the most predominant air pollutants today. The gas is highly soluble in water:  $113\text{g l}^{-1}$  compared to approximately  $1.69\text{g l}^{-1}$  for oxygen, nitric oxide, carbon monoxide, and carbon dioxide (Parker, 1977).  $\text{SO}_2$  and its oxidative byproducts, sulfur trioxide and the corresponding acids and salts (sulfites and sulfates), are produced from the combustion of solid and liquid fossil fuels containing sulfur in some form. For example, the sulfur content in bituminous coal ranges from 0.3 to 5.0% and higher; most commonly, from 0.5 to 2.5%. About 80% of the sulfur in coal, and nearly all that in liquid and gaseous fuels, appears in flue gases in the form of  $\text{SO}_2$ . The actual amount of  $\text{SO}_2$  found in the air varies from almost nil in some areas to about 3 ppm in heavily industrialized areas (Ziegler, 1975).

Another common source of  $\text{SO}_2$  in the atmosphere is metallurgical operations (Figure 1). Many ores, e.g., zinc and copper, are primarily sulfides. During the smelting of these ores,  $\text{SO}_2$  is produced at stack concentrations of 5 to 10%.

Due to the extremely high concentration of  $\text{SO}_2$  found in many industrial stacks, methods have been developed to abate this problem. For example, where the concentration of  $\text{SO}_2$  is of the order of 5 to 10%, the sulfur content may be recovered economically from the stacks in the form of  $\text{H}_2\text{SO}_4$ .

.....

Figure 1. A common source of  $\text{SO}_2$  in the atmosphere is metallurgical operations (Kennecott Copper Smelter, Magna, Utah).





The movement of  $\text{SO}_2$  from point source may occur by: precipitation, atmospheric motions, and deposition on surfaces among other processes (Oke, 1978; Hill, 1971; Martin, 1980; Shair, 1982).  $\text{SO}_2$  has been measured in the plume of a remotely situated smelter (Mount Isa, Australia) at distances of up to 1,000 km from the source (Williams et al., 1981).

#### Plants - Sinks for $\text{SO}_2$

Research into the effects of  $\text{SO}_2$  on vegetation has been conducted for many years; excellent reviews on the topic have been published since 1951 (Barrett and Benedict, 1970; Heath, 1980; Ziegler, 1975; Thomas, 1951). Much of the early work was initiated by industries interested in developing formulae to compensate growers for crop damage resulting from their  $\text{SO}_2$  emissions (Brisley and Jones, 1950; Brisley et al., 1959). In more recent years, many studies have been initiated as a result of environmental concern.  $\text{SO}_2$  has been shown to cause harm in the biosphere to both animals and plants (Parker, 1977).

Since vegetation covers 90% of the Earth's land area, pollutant deposition on foliar surfaces can be considered as an important sink (Hill, 1971). The earliest recorded air pollutant effects on vegetation were related to the sulfur oxides (Heck and Brandt, 1977).  $\text{SO}_2$  is the major agent among the sulfur oxides causing injury to vegetation, although plants are injured to some extent by other sulfur compounds such as sulfuric acid aerosols (Heck and Brandt, 1977). Today, complete destruction of vegetation near point sources rarely occurs because of reduced  $\text{SO}_2$  emissions due to environmental

resolutions. However, vegetation is still severely injured around point sources.

Sulfur, is the fourth most important plant nutrient ranking behind nitrogen, phosphorus, and potassium. It has a major role in the synthesis of both proteins and chlorophyll. Plants usually receive adequate amounts of sulfur from the degradation of manure and other organic matter or through the direct application of fertilizers. Under limiting supplies of sulfur exposure to low concentrations of  $\text{SO}_2$  could be beneficial rather than detrimental (Thomas et al., 1943; Cowling et al., 1973; Maugh, 1979).

Atmospheric  $\text{SO}_2$  taken up from the atmosphere may affect vegetation in two general ways: (a) Various forms of precipitation are effective in transporting  $\text{SO}_2$  from the atmosphere to the soil where it contributes to the sulfur supply and is available to plant roots (Faller, 1972; Cowling et al., 1973; Maugh, 1979;), (b)  $\text{SO}_2$  may be absorbed directly through the leaves (Olsen, 1957).

$\text{SO}_2$  injures vegetation when large quantities of the gas are absorbed by the leaves. In the humid mesophyll environment of the leaf, the gas reacts with water to form the highly toxic sulfite ion (Thomas and Hendricks, 1956). Sulfite then oxidizes to sulfate.

Oxidation of  $\text{SO}_2$  to sulfate may be of considerable importance as a mechanism of detoxification because it is approximately 30 times less toxic than  $\text{SO}_2$  (Thomas et al., 1943). However, sulfate is toxic at high concentrations (Silvius et al., 1975). Usually only a small portion of sulfur is assimilated into the organic portion, e.g., cysteine, cystine, and methionine (Thomas et al., 1943).

The effects of these sulfur compounds can be viewed as either visible or subtle. Both visible and subtle effects are induced by physiological and biochemical changes within the plant systems. Visible injuries are identified from morphological, pigmented, chlorotic, and/or necrotic foliar patterns that result from major physiological disturbances in plant cells. Subtle effects are those that do not result in visible damage but cause measurable changes in the growth patterns or physiology of the plants. A number of workers have reported on the injurious effects of  $\text{SO}_2$  on photosynthetic pigments (Roa and Le Blanc, 1965; Asada et al., 1968; Malhotra, 1977;), growth and yield reductions (Karnosky, 1976; Sisson et al., 1981), and the inhibition of several processes of plant metabolism (Reviewed by Ziegler, 1975).

Photosynthesis and carbon allocation. Photosynthesis (the production of photosynthates) and carbon allocation (the transport and partitioning of these photoassimilates) are vital to the energy budget of the plant. Limited availability of carbohydrates due to an alteration of carbon assimilation, metabolism, or allocation may result in lower productivity. For example, depressed assimilation rates have been reported for plants exposed to  $\text{SO}_2$  under various experimental regimes (Sij and Swanson, 1974). Taniyama and co-workers (1972) found a decrease in dry matter production in rice exposed to  $\text{SO}_2$  which was attributed to depressed photosynthetic rates with increased respiration rates.

It has been shown repeatedly that  $\text{SO}_2$  has either a temporary or lasting inhibitory effect on photosynthetic assimilation, the duration being a function of interval and level of exposure, age of exposed tissue, and environmental conditions (Sij and Swanson, 1974; Black and Unsworth, 1979; Sisson et al., 1981;).

The mechanism of  $\text{SO}_2$  action on photosynthesis is not known, although several plausible explanations exist (Ziegler, 1972; Hallgren, 1978; Heath, 1980). The competitive inhibition of ribulose biphosphate (RuBP) carboxylase by  $\text{SO}_3^{-2}$  with respect to  $\text{HCO}_3^-$  ( $\text{CO}_2$ ) (Ziegler, 1972) has been considered a key mechanism through which the byproducts of  $\text{SO}_2$  directly interfere with  $\text{CO}_2$  fixation. However, Gezelius and Hallgren (1980) have shown that RuBP carboxylase was noncompetitively inhibited by  $\text{SO}_3^{-2}$  with respect to  $\text{CHO}_3^-$ . Other investigators have suggested that the inhibition of photosynthesis may be explained by non-specific alteration of membrane integrity (Luttge et al., 1972), by inhibition of oxygen evolution (Silvius et al., 1975) and electron transport over photosystem II (Shimazaki and Sugahara, 1980), by uncoupling of photophosphorylation (Sij and Swanson, 1974), or by effects of enzymes involved in different pathways (Pahlich, 1975; Horsman and Wellburn, 1976; Zielger, 1975; Pierre, 1977; Rabe and Kreeb, 1980; Malhotra and Kahn, 1980).

Sucrose, the major product of photosynthesis is synthesized in the cytoplasm of mesophyll cells from products of the reductive photosynthetic carbon cycle in the chloroplasts. Sucrose is transported through the phloem tissue to different sink<sup>1</sup> regions within the plant.

Very little is known about the effects of  $\text{SO}_2$  on carbohydrate translocation in plants. Recent evidences suggest greater reductions in translocation than that expected when beans were exposed to 0.1-3.0 ppm  $\text{SO}_2$  (Noyes, 1980; Teh and Swanson, 1982). They hypothesized that under  $\text{SO}_2$  exposure, a direct effect on the sucrose carrier protein may occur, making it inoperatable, thus, reducing translocation.

Nitrogen fixation.  $\text{N}_2$ -fixation occurs in a unique symbiotic relationship. It is found among lichens (blue-green algae living with fungi) between certain trees (Alnus and Pseudotsuga) and grasses (Digitaria and Paspalum). The most well-known example is the obligatory symbiotic  $\text{N}_2$ -fixation by Rhizobium spp. bacteria in association with leguminous angiosperms such as alfalfas, clovers, snapbeans, and soybeans.

The role  $\text{N}_2$ -fixing organisms serve in recycling atmospheric nitrogen has long been recognized by man. Nodulated legumes grown for grain, hay, pasture, and other agricultural purposes account for almost half ( $80 \times 10^6$  metric tons) of the annual quantity of nitrogen fixed by biological systems (Hardy and Havelka, 1975).

Active  $\text{N}_2$ -fixing nodules are strong sinks for photoassimilates. Thus, the amount of available sucrose may limit  $\text{N}_2$ -fixation. A sufficient knowledge of  $\text{SO}_2$ 's affects of  $\text{N}_2$ -fixation is lacking.

Studies have shown that the rates of photosynthesis and  $\text{N}_2$ -fixation in lichens and algae decreased significantly after

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<sup>1</sup>Regions within the plant receiving photosynthates from source leaves predominately in the form of sucrose, are termed sink regions.

exposure to aqueous solutions of  $\text{NaHSO}_3$  and  $\text{NaHSO}_4$  (Hallgren and Huss, 1975; Sheridan, 1979). It has been suggested that the reduction of  $\text{N}_2$ -fixation was caused by direct action on the enzyme nitrogenase or indirectly, by affecting photosynthesis (Sheridan, 1979). Evidence supporting direct action on the nitrogenase enzyme was reported by Hallgren and Huss (1975) where an inhibition of  $\text{N}_2$ -fixation without a reduction in photosynthesis in both the lichen Stereocaulon paschale L. and the blue-green algae Anabaena cylindrica.

These above investigations were performed using lower organisms (e.g. lichens and algae). To date, only one paper has been found reporting the use of legumes. Sheridan (1979) fumigated soybeans with a  $\text{SO}_2$  air mixture for a period of 14 days and found a stimulation of  $\text{N}_2$ -fixation at the lowest concentrations (20-30 ppb) and inhibition at  $\text{SO}_2$  concentrations of 50 ppb or higher.

This study was initiated to test various hypotheses dealing with  $\text{SO}_2$ 's effect on  $\text{N}_2$ -fixation, carbon allocation and general productivity of bean. The null hypotheses that were tested are:

1. General productivity, growth and yield, will not significantly change in plants exposed to  $\text{SO}_2$  fumigation because of a combination of experimental factors--low  $\text{SO}_2$  concentrations, short fumigation intervals, and the stage of ontogeny at time of exposure, all of which result in short-term effects.
2.  $\text{SO}_2$  will not alter carbohydrate ( $\text{C}^{14}$ ) partitioning ratios within the plant.

3.  $\text{SO}_2$  will not reduce  $\text{N}_2$ -fixation (acetylene reduction) in the nodules because of a reduction in photosynthate supply.
4. If acetylene reduction rates are decreased, they will not recover after the treatment has been terminated as a function of exposure time.



## MATERIALS AND METHODS

General Procedure

Snapbeans (Phaseolus vulgaris L.) were grown in a temperature controlled greenhouse (July-October 1982). On the 29th day from planting, plants were exposed to 0, 0.4 and 0.8 ppm  $\text{SO}_2$ . These concentrations were selected because they would interfere with metabolism without visible injury.

Plants were treated with  $\text{SO}_2$  at the same time each day for 4 hours for 5 consecutive days. Chamber levels of  $\text{SO}_2$  relative humidity, and temperature were monitored continuously during the treatment period.

Before terminating the fifth day fumigation, plants were pulse-labeled with  $^{14}\text{CO}_2$  to determine relative photosynthetic rates and  $^{14}\text{C}$  allocation patterns.

During the three days post-treatment, six plants were randomly sampled from the 24 plants of the treatment population and various parameters of growth, carbon allocation and acetylene rates recorded. Upon maturity (70 days) the remaining plants were sampled for yield determinations.

Plant Material

Seeds of snapbeans (Phaseolus vulgaris L. cv. Earliwax) were inoculated with Rhizobium phaseoli L. and cultured in a dark sand-peat mix (7:3 respectively) using 15 cm black plastic pots. All plants were grown to maturity in a temperature controlled greenhouse (Logan, Utah). Supplemental florescent light ( $300 \mu\text{E s}^{-1} \text{ m}^{-2}$ ) was

given to extend the photoperiod to 16 hours. The temperature and relative humidity were measured and recorded using a hydro-thermograph (Weather Measure Corp. Model #H311) that averaged  $26 \pm 3^\circ\text{C}$  and  $40 \pm 5\%$  RH respectively. All plants received half-strength Hoagland's solution (Hoagland and Aarson, 1950), minus nitrogen, 3 days a week, with alternate irrigation of tap water when needed.

After emergence of the seedlings, the pots were thinned to two plants of similar size. At the onset of fumigation, 29-day old plants were chosen from a large population to further assure a better representation of similar developmental stages rather than merely being of the same chronological age. Each  $\text{SO}_2$  treatment consisted of 24 plants.

Harvesting of plant material at post-fumigation was performed at the greenhouse. Plants were removed from their respective pots, roots gently rinsed on a wire mesh screen positioned over a bucket to remove most of the soil particles (Figure 2 and 3). Each plant was immediately placed in separate plastic bags and taken to the laboratory for analysis. A maximum of 20 minutes lapsed between the time of washing and arrival at the laboratory.

#### $\text{SO}_2$ Fumigation Chamber

Specific concentrations of  $\text{SO}_2$  were maintained in a greenhouse-situated fumigation chamber (Figures 4 - 6) built with clear acetate plastic. The chamber, 122 cm wide by 70 cm high by 92 cm deep ( $786 \text{ cm}^3$ ), rested on a 1.3 cm sheet of plywood atop an east greenhouse bench 100 cm off the floor. All seams within the chamber were sealed air tight with silicon cement. A removable door (40 cm by



Figure 2. A nodulated snapbean plant 33 days from planting.





Figure 3. A nodulated snapbean root system 33 days from planting.

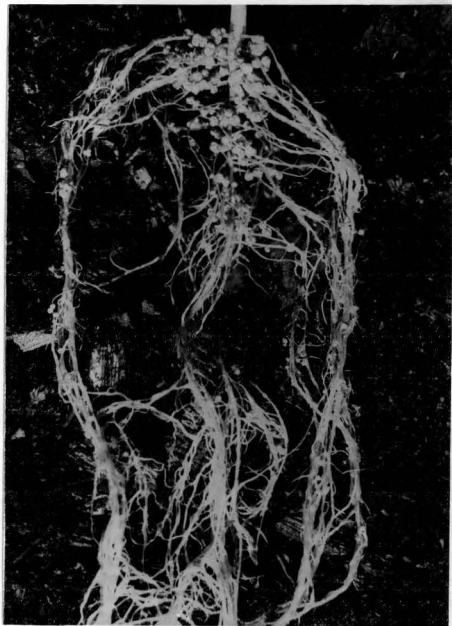






Figure 4. All plants were exposed to  $\text{SO}_2$  in a greenhouse situated clear acetate plastic fumigation chamber.

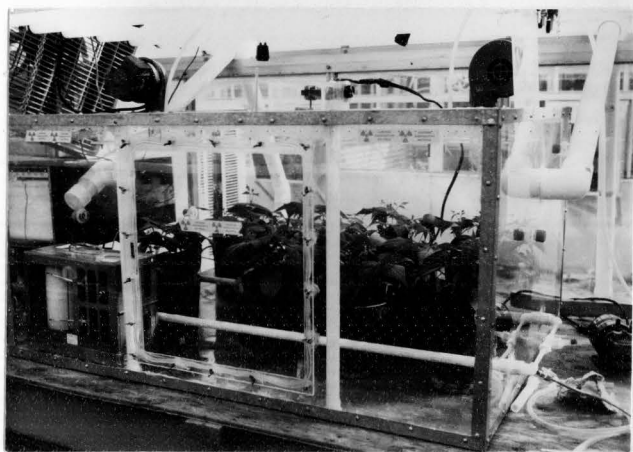




Figure 5.  $\text{SO}_2$  fumigation chamber (side and front views).

Key

1. Air influx port
2.  $\text{SO}_2$  influx port
3. Deflection shield
4.  $\text{SO}_2$  sampling system
5.  $\text{SO}_2$  sampling port with rubber septum
- 5a. Additional  $\text{SO}_2$  sampling port
6. Exhaust port extending to the outside of the greenhouse
7. Circulation fan
8. Rheostat for circulation fan
9. Chamber door sealed with a swing nut-bolt system and "O" ring
10. Thermometer (bulb type) and cup shield

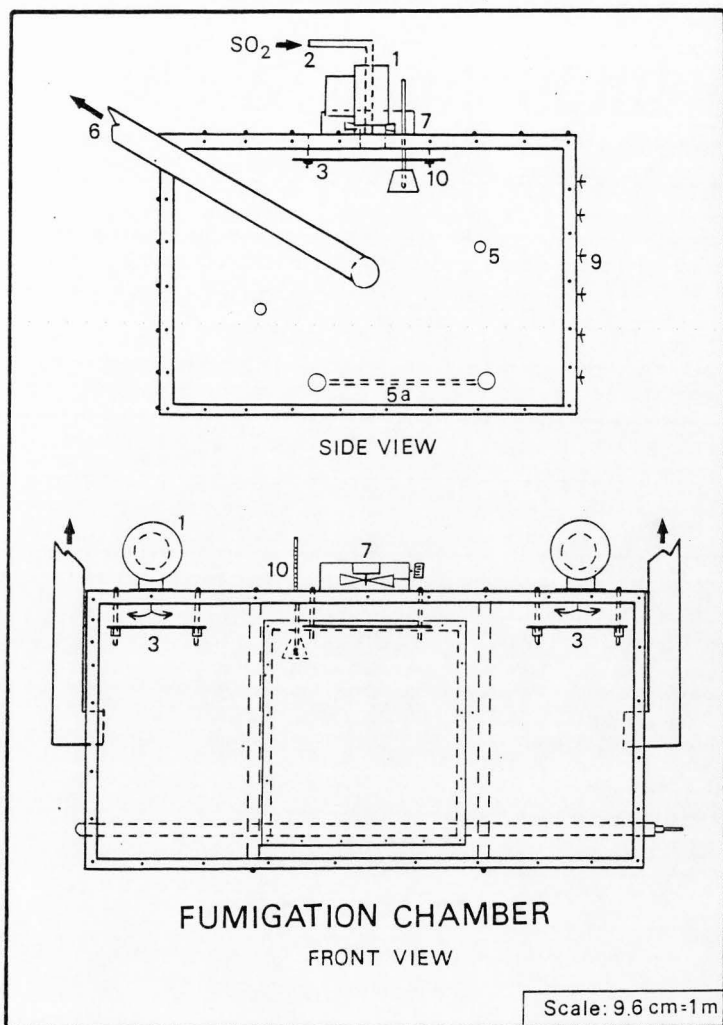


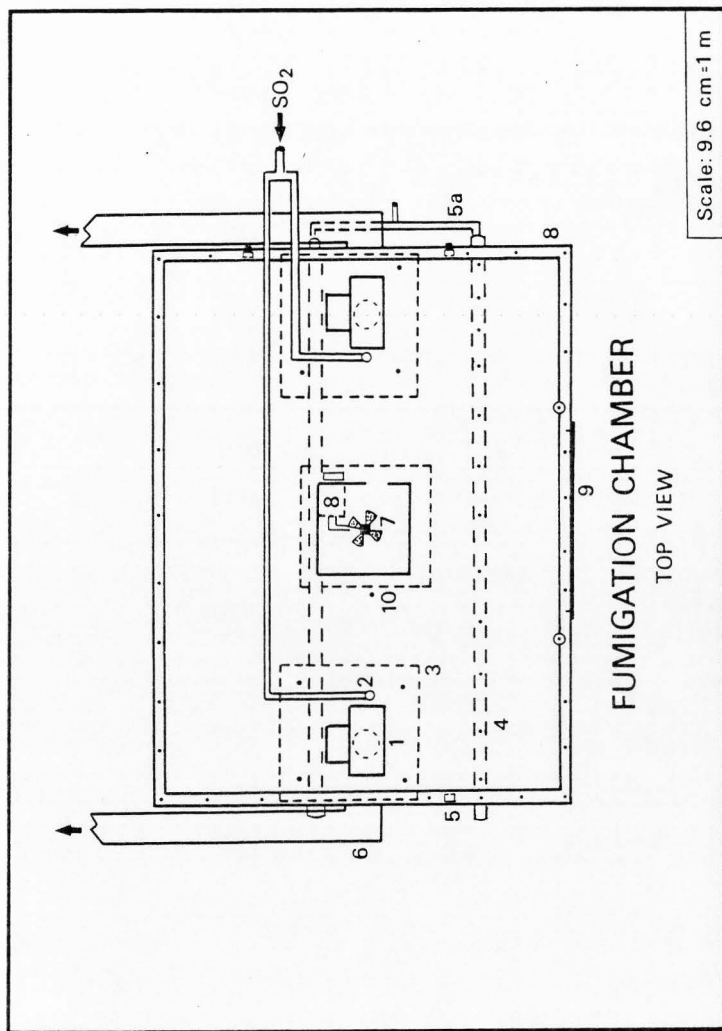


Figure 6.  $\text{SO}_2$  fumigation chamber (top view).

Key

1. Air influx port
2.  $\text{SO}_2$  influx port
3. Deflection shield
4.  $\text{SO}_2$  sampling system
5.  $\text{SO}_2$  sampling port with rubber septum
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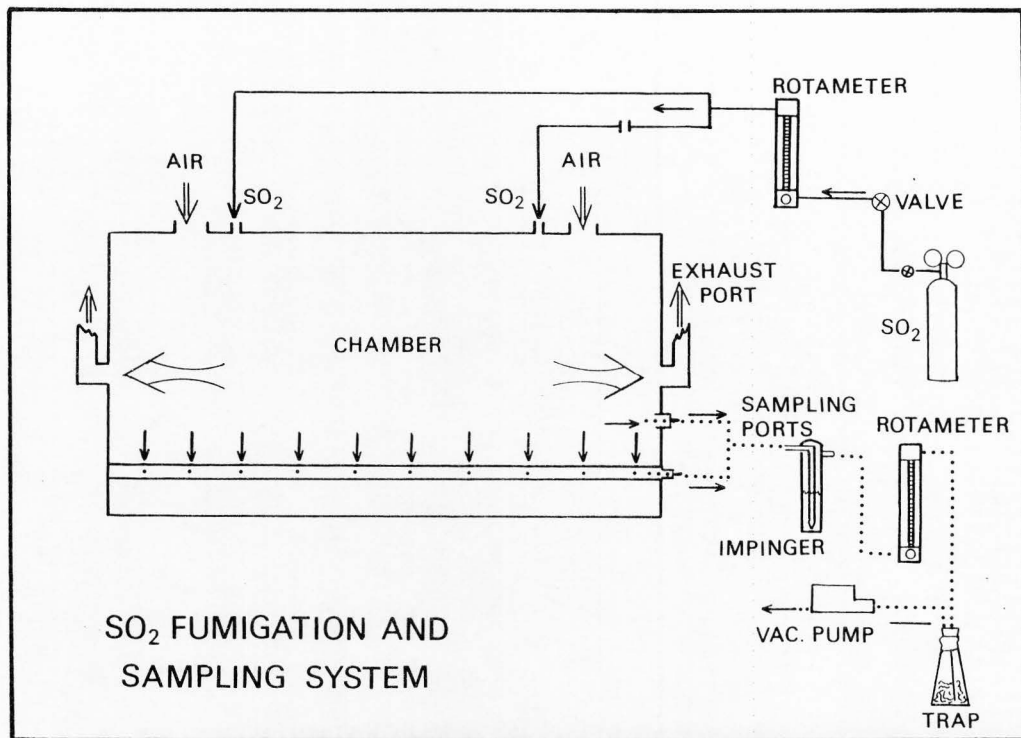


45 cm) was placed in front and tightly secured with the addition of a Tygon "O" ring and a wingnut-bolt system inserted every 10 cm.

The amount of  $\text{SO}_2$  released into the chamber was regulated to obtain the desired  $\text{SO}_2$  concentration (Figure 7). The  $\text{SO}_2$  was controlled by a needle valve and calibrated flow meter (Lab Crest Model #10A1460) from a stock tank of 1%  $\text{SO}_2$  (Matheson Co) to the fumigation chamber through 1 cm Tygon tubing inserted into the chamber roof. Air was forced into the chamber by two roof mounted 7.62 cm electric squirrel cage fans. Total air influx from the dual fan system was in the range of approximately  $2,750 \text{ l min}^{-1}$  ( $45.83 \text{ l sec}^{-1}$ ) at an internal air pressure of 0.65 cm of water. This gave a chamber  $\text{SO}_2 \text{ air}^{-1}$  exchange rate of approximately three times a minute. The  $\text{SO}_2 \text{ air}^{-1}$  mixture was circulated within the chamber by a roof situated electro-magnetic circulating fan (7.62 cm diameter) and rheostat control. A deflection shield was placed in front of each fan and blower assembly for better dispersion of gases ( $\text{SO}_2 \text{ air}^{-1}$ ) within the chamber. The efflux of  $\text{SO}_2 \text{ air}^{-1}$  from the chamber was achieved by means of two 5 cm polyethylene vinyl (PVC) portals connected to a PVC pipe system exiting to the outside of the greenhouse. Two glass sampling portals, sealed with rubber septa, were installed on each of the three walls of the chamber for easy  $\text{SO}_2$  sampling. Artificial lighting was positioned directly over the fumigation chamber (consisting of eight Sylvania F40CW fluorescent lights) to provide a consistent lower limit of photon flux and to compensate for shading caused by various greenhouse obstacles (e.g. overhead steam pipes).



Figure 7.  $\text{SO}_2$  fumigation and sampling systems.



### Fumigant Sampling and Analysis

Measured volumes of the  $\text{SO}_2$   $\text{air}^{-1}$  mixture from within the chamber were withdrawn and assayed using a widely used colorimetric method (West and Gaeke, 1956).

To obtain a  $\text{SO}_2$   $\text{air}^{-1}$  sample for analysis, 30 l of  $\text{SO}_2$   $\text{air}^{-1}$  was withdrawn from the chamber (Figure 7) through 10 ml solution of tetrachloromercurate (TMC) solution contained in a 30 ml glass impinger. Previous studies (Scaringelli et al., 1967; Reiszner and West, 1973) indicated that the 10 ml TMC solution was sufficient for collection of all  $\text{SO}_2$  in an air sample. Each sampling of  $\text{SO}_2$   $\text{air}^{-1}$  was withdrawn using a vacuum pump (Neptune DYNA-pump model #4K) for 60 minutes with a flow rate of  $0.5 \text{ min}^{-1}$ . The flow rate through each impinger was measured by a calibrated rotameter. During each fumigation interval ( $4 \text{ h day}^{-1}$ ) the air sampling for  $\text{SO}_2$  determination was replicated four times. After the sampling interval was completed, the exposed TCM solution was stored at  $4^\circ\text{C}$  for later analysis using the West-Gaeke Method (1956) as modified by Scaringelli et al: (1967). Upon analysis the samples were reacted with pararosaniline and formaldehyde to form intensely colored pararosaniline methyl sulfonic acid. The absorbance of the sample solution was read spectrophotometrically at 548 nm wavelength.

### Acetylene Reduction Assay

The Specific Nodule Activity (SNA) of each plant was determined by following the acetylene ( $\text{C}_2\text{H}_2$ ) reduction assay outlined by Hardy et al. (1968). This involved monitoring the reduction of acetylene to

ethylene ( $C_2H_4$ ) by gas chromatography. Whole excised nodulated roots, washed and gently blotted dry, were placed in 250 ml, wide mouth, Nalgene plastic bottles. A 10% volume of air was then removed and replaced with the same volume of acetylene. After one hour incubation time, a 500  $\mu$ l sample was withdrawn from each bottle and assayed for reduction of acetylene to ethylene. The reduction assay was performed on a Hewlett-Packard Model 5880A gas chromatography. The separation of the sample gases, acetylene and ethylene, was accomplished with porous cross-linked polymer beads (Porapak: Type N, mesh 100-200) designed to produce separations for  $C_1$ - $C_4$  hydrocarbons. These were packed into a glass column 2m x 2mm (ID = internal diameter) x 6 mm (OD = outer diameter).

Ethylene standards were injected and programmed as a standard curve into the gas chromatograph. The data, along with nodule dry weight measurements, were used to calculate SNA ( $\mu$  mol  $C_2H_4$  h<sup>-1</sup> mg dry weight<sup>-1</sup>).

#### Growth and Yield Measurements

All plant material was separated into nodule, root, leaf, stem, and bud (flower and leaf buds). Plant parts were oven dried (70°C for 24 hours) and dry weight (mg plant<sup>-1</sup>) were recorded. The leaf area (cm<sup>2</sup>) of all leaves plant<sup>-1</sup> were measured using a leaf area meter immediately after excision from the stem. Total nodule-root and stem-leaf-bud weights were used to calculate root shoot<sup>-1</sup> ratios. Yield components were harvested at plant maturity and separated into the number of pods plant<sup>-1</sup>, number of seeds pod<sup>-1</sup>, and dry weight of seeds recorded.

### Carbon Partitioning

One hundred minutes before the conclusion of the five day treatment, 33-day old snapbeans were pulse labeled with  $^{14}\text{CO}_2$ . The pulse-chase lasted for 10 and 90 minutes, respectively. The production of  $^{14}\text{CO}_2$  took place within a specially designed flask where 100  $\mu\text{l}$   $\text{Na}_2^{14}\text{CO}_3$  (specific activity 2.5 mCi  $\text{ml}^{-1}$ ) (New England Nuclear) was volatilized by addition of 4 N HCl. The total  $^{14}\text{CO}_2$  evolved was captured within a 50 ml syringe and injected into the fumigation chamber via side wall sampling ports fitted with rubber septa. Due to the mid-day (1120 hours) greenhouse environmental conditions at the time of the  $^{14}\text{CO}_2$  pulse, the chamber blower was switched on periodically to alleviate overheating within the chamber. As a result, known concentrations of  $^{14}\text{CO}_2$  were not maintained and, therefore, relative photosynthetic rates could not be calculated. The mean internal chamber temperature and relative humidity during this chase period were  $28.8 \pm 0.3^\circ\text{C}$  and  $39.9 \pm 0.6\%$  respectively. The photon flux (400-700 nm) averaged 500  $\mu\text{E s}^{-1}\text{m}^{-2}$ . At the conclusion of the 10 minute  $^{14}\text{CO}_2$  pulse, the chamber blower was switched on and remained running until the termination of the  $\text{SO}_2$  treatment (90 minutes later). The  $\text{SO}_2$  influx was turned off just prior to administering the  $^{14}\text{CO}_2$  injection and switched on immediately following the 10 minute pulse.

When the  $\text{SO}_2$  treatment was completed, the plants were removed from the chamber and placed on the greenhouse bench. Six plants were chosen at random to determine  $^{14}\text{C}$  partitioning. Sampling continued



for the succeeding 2 days at 24 hour intervals, to determine  $\text{SO}_2$  effects on whole plant growth patterns.

Plant material was fractionated into nodules, roots, stems, leaves, and buds then oven dried and weighed, and  $^{14}\text{CO}_2$  treated plant material was ground in a Wiley mill (40 mesh) under a hood and the radioactivity of the subsamples were determined after digestion with  $\text{HClO}_4\text{-H}_2\text{O}_2$  (two parts 70% perchloric acid to three parts 30% hydrogen peroxide). The sample was then tightly capped and placed in an oven ( $50^\circ\text{C}$  for 4 hours). After cooling, liquid scintillation cocktail (Aquasol-2, New England Nuclear) was added to each vial and radioactivity measured as disintegrations per minute (DPM) by a Packard Tri-Carb 460 CD liquid scintillation system.

The data were expressed as:

$$\text{Relative Sink Activity} = \frac{\text{DPM/mg dry weight of sink}}{\text{DPM/mg dry weight of total plant}}$$

$$\text{Relative Sink Strength} = \text{DPM of sink} \times \text{Relative Sink Activity}$$

### Statistical Analysis

Data were subjected to an analysis of variance (Table 2) using a split-plot design with subsampling. Three replicates of the main plot ( $\text{SO}_2$  treatment) were arranged in a randomized block fashion composed of three different 1 week fumigation treatments (0.0, 0.4, 0.8 ppm  $\text{SO}_2$ ) using a single fumigation chamber. Split-plots were divided into 0, 1, 2 days post-fumigation periods. Samples and subsampling consisted of three pots and two plants  $\text{pot}^{-1}$  respectively. F values

were used to identify significance (0.05 level) at both the main and sub-plot levels. LDS values were used to compute the least significant difference between treatment means.

Table 2. Analysis of variance: split-plot in time

| Sources of Variation                | Degrees of Freedom |
|-------------------------------------|--------------------|
| Replications                        | 2                  |
| SO <sub>2</sub><br>error (a)        | 2<br>4             |
| Days<br>error (b)                   | 2<br>4             |
| SO <sub>2</sub> x Days<br>error (c) | 4<br>8             |
| Pots/expt. unit                     | 54                 |
| Plants/Pot                          | 81                 |
| Total                               | 161                |

## RESULTS AND DISCUSSION

Initial experiments were conducted to determine the threshold for visible injury under the current experimental conditions. Visible injury to P. vulgaris occurred at 1.5 ppm  $\text{SO}_2$  during a one day 4 hour exposure period (Figures 8a&b). Since the objective of this study was to stay below the threshold of visible injury,  $\text{SO}_2$  treatment concentrations of 0.0, 0.4, and 0.8 ppm  $\text{SO}_2$  and fumigation intervals of 4 hour  $\text{day}^{-1}$  for 5 days were selected.

The stage of plant ontogeny chosen for treatment was pre-anthesis (bud clusters present but not opened) due to existence of an array of sinks competing for photoassimilates. Any effect of  $\text{SO}_2$  on carbon partitioning may alter sink activity. Under greenhouse conditions, the snapbeans reached pre-anthesis between 29 and 36 days (on an average) from the time of planting (Figure 9). Initial flowering was reached by day 36.

Growth and Yield

There were no significant differences in total dry weight, root  $\text{shoot}^{-1}$ , and leaf area between the  $\text{SO}_2$  main effects, at the split-level days, or with the split-level interaction between days and  $\text{SO}_2$ . Analysis of the yield data showed no significant differences between the number of pods  $\text{plant}^{-1}$ , number of seeds  $\text{pod}^{-1}$ , or dry weight among seeds. These results support the hypothesis that general productivity, growth, and yield will not significantly change in plants subjected to short term low level  $\text{SO}_2$  exposures.



Figure 8a&b.  $\text{SO}_2$  fumigated snapbean plants showed signs of 1.5 ppm visible injury on the leaves after a 4 hour exposure to 1.5 ppm  $\text{SO}_2$  (B) - control plants (A).



3A

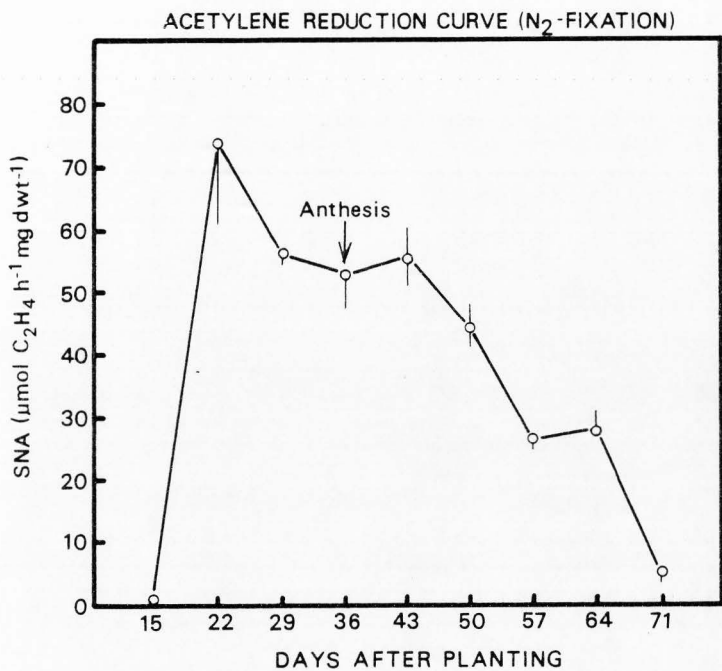


8B





Figure 9. Means and standard errors for the acetylene reduction (SNA) of snapbeans *P. vulgaris* L. cv. Earliwax from the time of planting to maturity. Bars denote SE of individual means.



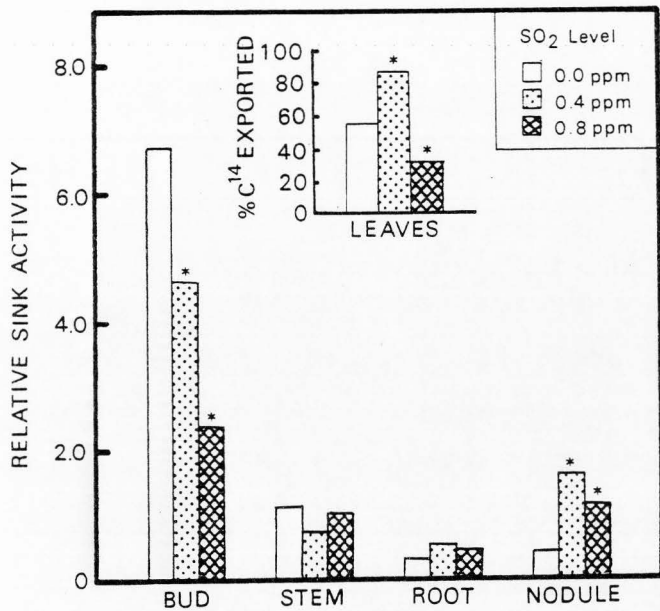
In most low level  $\text{SO}_2$  fumigation studies reductions in growth and yield were observed under longer  $\text{SO}_2$  exposure periods (Reinert and Sanders, 1982; Flagler and Youngner, 1982; Biggs and David, 1981; Lockyer and Cowling, 1981).

#### Carbon Partitioning

Export of  $^{14}\text{C}$  from the leaves was enhanced by an average of 34% in plants treated at 0.4 ppm  $\text{SO}_2$  for 4 hours  $\text{day}^{-1}$  for 5 days (Figure 10). This could be accounted for if photorespiration was suppressed.  $\text{SO}_2$  inhibition of photorespiration would result in a net increase of  $^{14}\text{C}$  fixation (Tolbert, 1980). The highly reactive byproduct of  $\text{SO}_2$  in solution is  $\text{SO}_3^{2-}$ . This chemical species can undergo addition reactions with cellular aldehydes and ketones producing a class of compounds referred to as  $\alpha$ -hydroxysulfonates. These addition products have been shown to be effective competitive inhibitors of glycolate oxidase (Tanaka et al., 1972; Zelitch, 1957). The inhibition of the photorespiratory pathway would bring about both a reduction in glycine and serine production and, at the same time, increasing  $^{14}\text{C}$  levels in the leaf. This has been demonstrated in a study using Lolium perenne exposed to  $\text{SO}_2$  levels similar to those chosen in this study (Kozioł and Cowling, 1978). Increases in  $^{14}\text{C}$  would then make more carbon available for export. At the 0.4 ppm  $\text{SO}_2$  level  $^{14}\text{C}$  loading may be hindered but not to the extent found at 0.8 ppm  $\text{SO}_2$  level as evidence has shown (Milchunas et al., 1982). The increase in net carbon assimilation due to a reduction in photorespiration might compensate for the reductions in the translocation rates.



Figure 10. The effects of short-term  $\text{SO}_2$  exposures on the export of  $^{14}\text{C}$  from leaves and specific sink activity (\* = significance at  $\alpha = 0.05$ ).



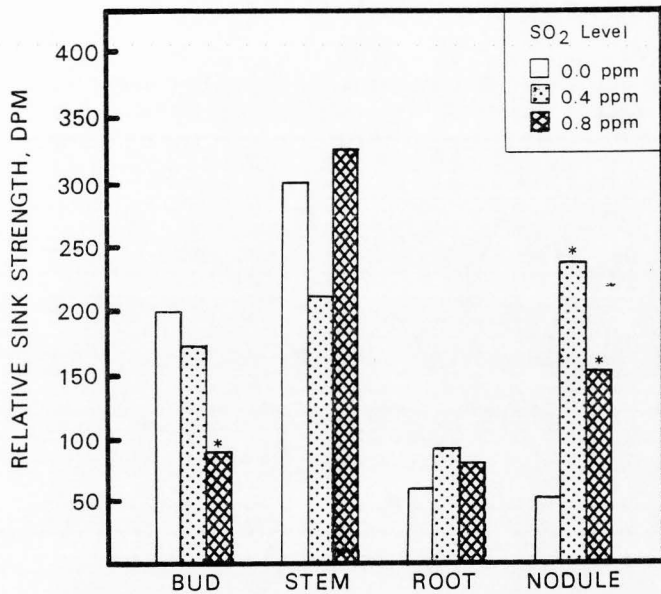
Plants exposed to 0.8 ppm  $\text{SO}_2$  for 4 hours  $\text{day}^{-1}$  for 5 days exported on an average of 20% less  $^{14}\text{C}$  from their leaves (Figure 10). At this higher treatment level, photorespiration may also be inhibited, but  $\text{SO}_2$  may be playing a stronger role in inhibiting phloem loading. Inhibition of [ $^{14}\text{C}$ ] sucrose loading into the minor veins from the apoplast has been demonstrated previously (Kozioł and Jordan, 1978; Noyes, 1980; Teh and Swanson, 1982). Luttge et al. (1982) concluded in his study that bisulfite compounds are inhibitors of many membrane functions, such as: ATP formation in photosynthesis and respiration,  $\text{H}^+$  fluxes, and  $\text{Cl}^-$  transport.  $\text{SO}_2$ , having access to the apoplast of leaf tissue, may have an adverse effect on the proposed plasmalemma sucrose/ $\text{H}^+$  carrier (Giaquinta, 1979), thus limiting sucrose export from the leaf (Noyes, 1980; Teh and Swanson, 1982). Noyes (1980) found a reduction in translocation without a reduction in photosynthesis at  $\text{SO}_2$  concentrations of 0.08, 0.25, and 0.86 ppm using Phaseolus vulgaris. Teh and Swanson (1982) reported that photosynthesis and translocation were inhibited by 74 and 48%, respectively after a 2 hour exposure to 3.0 ppm  $\text{SO}_2$ . Evidence using autoradiography showed that much of the  $^{14}\text{C}$  activity remaining was in, and accumulated around, minor veins of leaves exposed to  $\text{SO}_2$  suggesting that the phloem loading was hindered (Noyes, 1980).

The Relative Sink Strength (RSS) and Relative Sink Activity (RSA) (Figure 10) data exhibited a similar pattern among  $\text{SO}_2$  treated plants. RSA refers to the "mobilizing ability" to "attract" assimilate whereas RSS would refer to the capacity to "accumulate" assimilates. Exposure to  $\text{SO}_2$  resulted in a decline of both the RSA and RSS of the buds





Figure 11. The effect of short-term  $\text{SO}_2$  exposures on relative sink strength (\* = significance at  $\alpha = 0.05$ ).



(Figure 10 and 11) with a corresponding increase of the RSA and RSS of root-nodules. This shift of  $^{14}\text{C}$  allocation, resulting in decreased porportion of  $^{14}\text{C}$  in the buds and greater porportion in the nodules, may be due to lower  $^{14}\text{C}$  demand by the buds. Lower bud sink activity could result if accumulations of  $\text{SO}_2$  byproducts in the bud tissue occurred (Garsed and Mochrie, 1980).

The translocation of sulfur products, via the phloem, has been demonstrated after fumigation with  $^{35}\text{SO}_2$  (Garsed and Read, 1974) or application of droplets of  $^{35}\text{SO}_4^{2-}$  (Biddulph et al., 1956) or  $^{35}\text{SO}_3$  (Garsed and Mochrie, 1980) to the leaf surface. In all cases, the sulfur accumulated preferentially in the meristematric regions, such as buds, which may reduce their sink activity.

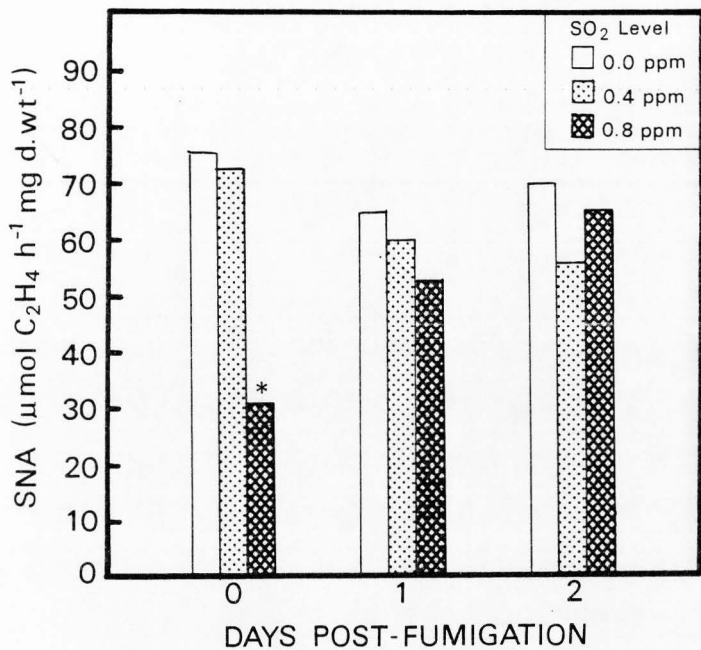
#### $\text{N}_2$ -Fixation (Acetylene Reduction)

The effect of  $\text{SO}_2$  on acetylene reduction (AR) among nodulated snapbeans is depicted in Figure 12. Acetylene reduction, expressed as SNA, among the 0.4 ppm  $\text{SO}_2$  treated plant showed no significant difference from controls. The SNA of the 0.8 ppm  $\text{SO}_2$  treated plants was significantly reduced (59%) from controls at termination of the fumigation treatment. A recovery of SNA of 0.8 ppm  $\text{SO}_2$  treated plants to control levels occurred by day 2 (48 hours) post-treatment.

Reduction in  $\text{N}_2$ -fixation by  $\text{SO}_2$  exposure in this study are consistant with the only other study known using legumes (Sheridan, 1979). Sheridan (1979) exposed soybean plants to  $\text{SO}_2$  for 14 days and found a 25, 60 and 75% reduction in  $\text{N}_2$ -fixation at 0.05, 0.1 and 1.0 ppm  $\text{SO}_2$  respectively. No mention of recovery rates was reported by Sheridan (1979). Hallgren and Huss (1975) found that  $\text{N}_2$ -fixation was



Figure 12. Effects of  $\text{SO}_2$  exposure on the SNA of snapbean root-nodules at 0, 1 and 2 days post-fumigation (\* = significance at  $\alpha = 0.05$ ).



inhibited in lichen and blue-green algae exposed to  $\text{NaHSO}_3$ . After removal from  $\text{NaHSO}_3$ ,  $\text{N}_2$ -fixation fully recovered within 8 hours.

From the results of this study, it would be statistically valid, at the 5% level, to accept the hypothesis that  $\text{SO}_2$  can reduce acetylene reduction in snapbean nodules. Under the prescribed experimental conditions outlined, the recovery to near control rates occur with time when plants have been exposed to low doses of  $\text{SO}_2$  for short periods.

How does  $\text{SO}_2$  reduce  $\text{N}_2$ -fixation?  $\text{N}_2$ -fixation may be inhibited because the root-nodules are lacking in available photoassimilates. Inhibition of phloem loading (Noyes, 1980; Teh and Swanson, 1982), reduced photosynthesis (Ziegler, 1972; Bennett and Hill, 1973; Sij and Swanson, 1974; Silvius et al., 1975; Sisson et al., 1981; Hallgren and Gezelius, 1982), greater sink strength and activity in other competing organs, other than the root-nodules (e.g., reproductive organs) (as seen in this study), inhibition of photorespiration (Kozioł and Cowling, 1978), increased stomatal resistance (Majernik and Mansfield, 1970; Unsworth et al., 1972; Sisson et al., 1981), or increased respiration rates (Kozioł and Jordan, 1978) may all contribute to decreased  $\text{N}_2$ -fixation. My data suggest (Figure 10 and 11) that root-nodules are not good competitors for available carbon as compared to buds and stem tissue. This may also be a proximity (buds closer to source) phenomenon. However, present data may not indicate carbon starvation to nodules as an explanation for reduced SNA as a result of  $\text{SO}_2$  treatment. Therefore an alternative hypothesis is offered.

Inhibition of  $N_2$ -fixation in plants exposed to  $SO_2$  may be explained through the possible existence of a regulatory mechanism controlling  $N_2$ -fixation by "feedback control" operating via the transpirational stream. If the supplies of nitrogenous compounds saturate a plant's system due to the plant's inability to metabolize or export these compounds, it can be assumed that the organism would indicate a mechanism(s) that would override the biosynthesis of these potentially toxic substances. No investigations have been found concerning this proposed hypothesis. If the phloem loading of sucrose is directly inhibited by  $SO_2$ , then phloem loading of amino acids (Servaites et al., 1979) in the leaves may also be inhibited. Therefore, the inability of these nitrogenous compounds to be cycled through the leaf may cause a feedback inhibition of  $N_2$ -fixation through a buildup of these compounds in the xylem.



## SUMMARY AND RECOMMENDATIONS

The capacity to reduce acetylene ( $N_2$ -fixation) among nodulated snapbean plants was decreased when the plants were subjected to a short-term  $SO_2$  fumigation (0.8 ppm). Upon the removal of  $SO_2$ , the SNA of the 0.8 ppm  $SO_2$  treated plants recovered to near control values within a two-day post-fumigation period. Although it can be demonstrated from this study that  $SO_2$  inhibits acetylene reduction, no statistically sound statements can be made as to the direct or indirect cause of this effect.

A close relationship between  $N_2$ -fixation and photosynthesis has been demonstrated (Hardy and Havelka, 1975, 1976). It was hoped initially that this study would demonstrate cause for the inhibition of  $N_2$ -fixation by measuring relative photosynthetic rates and photosynthate partitioning of treated snapbeans. It was hypothesized that  $SO_2$  would decrease photosynthate supplies to the root-nodules and, thus, deplete the nodules of necessary energy required to perform  $N_2$ -fixation.

Evidence from  $^{14}CO_2$  labeling experiments suggested that  $SO_2$  does affect  $^{14}C$  distribution among the various sinks of snapbean plants. More photosynthates were partitioned to the nodules of  $SO_2$  treated plants than control plants although an accurate assessment of the absolute quantity of  $^{14}C$  arriving at each sink cannot be determined here. Therefore, it is not known whether the quantities of  $^{14}C$  within the nodules of  $SO_2$  treated plants are increases in absolute amounts. To determine this, accurate measurements of the photosynthetic rates must occur. Due to the variability in the quantity of  $^{14}CO_2$  caused by

chamber constraints, measures of relative photosynthetic rates were unattainable. Only liberal speculation as to the cause of  $N_2$ -fixation inhibition can be deduced at this time.

At this point, the author would like to offer suggestions for improvement in the experimental design and present further questions for additional research.

#### Experimental Design

1. To Increase the Number of Chambers. Replications in the present study were conducted over a 3-week interval (1 week treatment<sup>-1</sup>) using one chamber. A minimum of three chambers (or 1 per treatment) should be included in the next design to alleviate the variation between treatments due to environmental changes occurring over extended periods.

2. Addition of Artificial Light Source. The addition of a more irradiant artificial light source above the chamber would reduce variability in photosynthesis caused by greenhouse shadowing and cloud cover changes during the treatment period.

#### Questions For Further Research

1. What is the cause of the reduction of  $N_2$ -fixation by  $SO_2$  treatment?

##### Possible Causes

a. Lack of sufficient carbohydrate supplies in the nodules due to the inhibition of photosynthesis and/or translocation and phloem loading?

b. Translocation of  $\text{SO}_2$  or its reductive byproducts reaching the nodules and accumulating to toxic levels thus inhibiting  $\text{N}_2$ -fixation?

2. What are the changes in absolute quantities of  $^{14}\text{C}$  at each sink under  $\text{SO}_2$  stress?

3. Are the buds affected metabolically by accumulations of sulfur compounds translocated via the phloem during  $\text{SO}_2$  exposures? If so, how does this correlate with sink activity in regard to photoassimilate demand?

4. Under low  $\text{SO}_2$  levels and longer exposure periods, is flowering delayed due to decreases in photoassimilate at the buds? Sink?

5. Is phloem loading of amino acids in the leaves of  $\text{SO}_2$  treated plants inhibited? If so, do these nitrogenous compounds accumulate and trigger a feedback inhibition on  $\text{N}_2$ -fixation?

One of the most difficult tasks following a study of this kind is relating the data to field conditions. Much more research is needed concerning  $\text{SO}_2$ 's effects on nitrogen fixation and carbon partitioning both in the laboratory and in the field before reliable correlations can be made. However, greenhouse investigations serve as a basis for further work. One drawback of field experiments without preliminary greenhouse studies is the inability to relate observations to known effects under somewhat "controlled" conditions. These data indicate that inhibition of  $\text{N}_2$ -fixation and the alteration of carbon allocation can occur under  $\text{SO}_2$  stress without visible injury to the above ground plant material. The importance of the fixed nitrogen contribution of

snapbeans, alfalfa, soybeans, and other legumes in both agricultural and natural habitats requires further considerations in respect to potential low level, long duration pollutant stresses placed on many legume species.

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